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## Immunity and vaccines against sexually transmitted *Chlamydia trachomatis* infection

Sarah E. M. Howie<sup>1</sup>, Patrick J. Horner<sup>2</sup>, Andrew W. Horne<sup>3</sup>, and Gary Entrican<sup>4</sup>

<sup>1</sup>MRC/University of Edinburgh Centre for Inflammation Research

<sup>2</sup>School of Social and Community Medicine, University of Bristol.

<sup>3</sup>Centre for Reproductive Biology, University of Edinburgh

<sup>4</sup>Moredun Research Institute and the Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh

### Abstract

**Purpose of review**—To review recent findings on immunity and vaccine development to *Chlamydia trachomatis*.

**Recent findings**—There is increasing knowledge on the interactions between *Chlamydia trachomatis* and infected host cells. During genital infection the organism avoids generating protective immunity but immune responses to a number of chlamydial proteins have been associated with reproductive tract pathology. Various vaccine and adjuvant preparations have been tried experimentally. Information generated by proteomics and complex studies of serological and T-lymphocyte immune responses points to novel vaccine candidates.

**Summary**—*Chlamydia trachomatis*, an obligate intracellular bacterium, is the commonest sexually transmitted infection worldwide and is associated with reproductive pathology. To develop rational vaccines it is necessary to understand the complex life-cycle of the organism, the host immune response to infection and how these relate to disease. Infection does not prevent reinfection and antibiotic treatment prevents antibody production at a population level. It remains unclear what type of immune response would be sufficient to prevent infection and/or reinfection. Although the prevalence and demographics of infection and the severity of disease associations suggest it would be desirable, there is no vaccine currently available. A number of studies have identified novel vaccine candidates.

### Keywords

*Chlamydia trachomatis*; Immunity; Vaccination

### The clinical problem - *Chlamydia trachomatis* and reproductive tract disease

*Chlamydia trachomatis* (Ct) is a gram-negative, obligate intracellular bacterium. Serovars A-C cause trachoma, D-K cause the common sexually transmitted infection (STI), L1-L2 cause lymphogranuloma venereum (LGV)[1]. This review focuses on immunity and vaccination against the common STI.

Prevalence of Ct STI in Europe is 1- 3% of the population between the ages of 18-44. Re-infection is common and most cases are asymptomatic[2]. It is accepted that Ct STI in some women leads to pelvic inflammatory disease (PID), tubal infertility and ectopic pregnancy although the evidence remains associative. There is also pathology associated with genital infection in men[3;4].

## **The host response to infection - Immunity and disease following *Chlamydia trachomatis* STI**

Since widespread antibiotic therapy was introduced serum anti-Ct antibodies have declined in parallel with increased Ct prevalence[5\*\*;6]. Whilst clearing infection, antibiotics may alter the development of immunity. It remains controversial whether pathology is caused directly by infection or by the immune response. There is evidence that women who make a pro-inflammatory, interferon- $\gamma$  (IFN- $\gamma$ ) dominated cytokine response (T helper Type 1/ Th1 response) have less re-infection and infertility than those who make IL-10 and IL-4 dominated Th2 responses[7\*;8\*]. As IFN- $\gamma$  induces a persistent non-infectious state in Ct in vitro[9\*;10] it remains unclear whether a Th1 response completely clears infection in vivo. Th1 dependent IgG2 antibodies to Ct antigens are higher in women with cervicitis and PID[11\*] indicating that Th1 responses may be associated with inflammatory pathology.

## **The potential solution - Is a vaccine likely to be beneficial?**

Modelling indicates that fully protective vaccination would eliminate Ct epidemics in 20 years. A partially effective vaccine would reduce disease in men and women, but cost-benefit would be greater for vaccinating women only[12\*]. To develop vaccines it is necessary to understand how Ct gains entry to and survives inside host cells; which bacterial proteins induce immune responses; and, which immune responses are protective.

Protective immunity generated by vaccination should not be confused with the immune response to STI. This is due to the context in which Ct antigens are seen by the immune system. In vaccination this is determined by the adjuvant(s) used and the route of administration. In natural infection Ct has evolved mechanisms to ensure that immune responses are suboptimal and/or are directed at proteins which are not essential for transmission or reinfection.

## **Complexity in miniature – the *Chlamydia trachomatis* lifecycle**

The Ct chromosome encodes nearly 1000 proteins. Most isolates have a plasmid encoding 8 or 9 proteins which influence bacterial gene transcription[13\*]. Ct has a bi-morphic life-cycle. The metabolically inactive, infectious, elementary bodies (EBs) have an extensively cross-linked outer membrane which makes them very stable extracellularly. The main structural components are the major outer membrane protein (MOMP/OmpA), OmcA and OmcB. Other proteins are involved in tropism and attachment. Heinz et al [14\*] used an in silico approach and Liu et al[15\*] used proteomics to identify several outer membrane proteins which may be targets for intervention strategies.

Inside permissive cells, EBs transition into rapidly dividing reticulate bodies (RBs) within host membrane bound inclusion bodies. Midway through the 48-72hr replication cycle some RBs transition to infectious EBs. The inclusion body expands and EBs are released by cell lysis or by extrusion of inclusion bodies. In vitro, persistent infection (blocking of RB to EB transition and generation of aberrant RB forms) results from exposure to penicillin, IFN- $\gamma$ , HSV infection and nutrient deprivation[9\*;10]. Skilton et al[16\*\*] demonstrated that penicillin removal from persistently infected cultures causes aberrant RBs to bud and form

normal RBs which then complete replication. Whether persistent infection occurs in vivo remains uncertain[9\*;16\*\*].

## Manipulating the environment - Pathogen induced host cell changes

Ct can use the cystic fibrosis transmembrane conductance regulator (CFTR) membrane protein to enter cells[17\*]. CFTR upregulation is associated with Ct induced female infertility[18\*]. EB entry depends on host membrane protein disulfide isomerase (PDI) [19\*] which targets cross-linked EB outer membrane proteins. PDI is a component of the estrogen receptor alpha (ERalpha) complex which associates with Ct attached to human endometrial epithelial cells[20]. Interaction with the ERalpha complex may be a target for prevention of infection as the co-receptor protein SRAP-1 is targeted by a Ct protease (CT441) to inhibit ERalpha signalling[21\*].

Inside host cells Ct circumvents endogenous stress mechanisms, prevents lysosomal fusion, inhibits apoptosis, and evades intracellular innate immunity. Cocchiari and Valdivia[22\*] reviewed intracellular survival mechanisms and Betts et al[23\*] reviewed how Ct effector proteins alter host cells. On entry, the EB protein TARP translocates into the cytoplasm, is phosphorylated by Src and remodels actin to allow the EB to move within the cell. The EB becomes surrounded by host cell membrane to form the inclusion body and Ct proteins are inserted into the inclusion body membrane. The inclusion body diverts and fuses with host cell vesicles containing nutrients necessary for replication. CPAF, a RB protease secreted into the cytoplasm, degrades cytoskeletal elements to allow inclusion body expansion and release of EBs; and processes intermediate-filament proteins to stabilize the inclusion bodies and minimize exposure to intracellular innate immune mechanisms[24\*]. CPAF also degrades pro-apoptotic proteins[24\*] and TARP interacts with the host adaptor protein SHC1 to inhibit apoptosis[25\*]. Caspase-1 activation promotes Ct replication[26\*]. Ct disrupts several signalling pathways[27\*]. In epithelial cells RAS decouples from MAPK/ERK (MEK) signalling although the effects differ in human and murine cells[28\*\*]. The Ct proteins ChlaDub1 and CT441 interfere with host cell NF- $\kappa$ B signalling[23\*].

## Fighting back - Immune responses to *Chlamydia trachomatis*

Each Ct protein may possess multiple antigenic epitopes that could be recognized by the acquired immune system. Some Ct molecules (proteins, LPS, lipids and CpG-oligonucleotides) have pathogen associated molecular patterns (PAMPS) which can be recognized by innate immune system receptors (PRRs).

## Avoiding innate immunity

Ct EBs have evolved to avoid innate immunity in the reproductive tract. Innate immune mechanisms include those triggered by membrane bound host PRRs, such as the Toll-like receptors (TLRs)[29] and NOD-1[30] which depend on NF- $\kappa$ B signalling. Although Ct LPS and proteins activate PRRs in vitro, responses to live infection are more muted suggesting that disruption of NF- $\kappa$ B signalling inhibits innate responses[31\*]. NF- $\kappa$ B was degraded in Ct infected human trophoblast cells [32\*].

Fallopian tubes from Ct infected women with ectopic pregnancy have increased inducible nitric oxide synthase/iNOS and increased pro-inflammatory molecules, activins, which are involved in scarring[33\*]. Thus inflammation may kill bacteria but chronic inflammation can cause tissue damage and this may partly explain why this study, like others, failed to detect Ct in diseased Fallopian tubes.

## Immunological markers of infection, pathology and protective immunity

Antibodies and T-lymphocyte responses to several Ct proteins have been described. Infection rate and organism load on reinfection decrease with age suggesting development of at least partial protective immunity[34].

Antibody responses require CD4-T-lymphocyte 'help'. B-lymphocytes and antibodies recognize 'native-protein' epitopes which are not necessarily linear, whereas T-lymphocytes recognise short linear peptides in the context of self MHC-I for CD8-T-cells and self MHC-II for CD4-T-cells. CD4-T-cells can be activated as pro-inflammatory Th17 and Th1 cells or as Th2 cells. Both Th1 and Th2 cells help B-cells to make antibody but the immunoglobulin subclasses produced differ. As different immunoglobulin subclasses vary in biological activity this may be relevant to pathology following infection.

A major antigenic source is the inclusion body. Ct encodes 50 inclusion body (Inc) proteins, 22 of which are in inclusion membranes[35\*]. Antibodies to Inc proteins are found in sera from infected women[11\*;35\*], and both CD4-[36\*] and CD8-[37\*] T-cells respond to Inc epitopes. Not all Ct proteins generate antibodies in every individual. Commercially available tests detect anti-EB antibodies by immunofluorescence or anti-MOMP antibodies by ELISA. Anti-EB antibodies were correlated with Fallopian tube damage in sub-fertile women[38]. ELISA assays suggest that Ct795[39\*], and the plasmid encoded Pgp3[40\*] may be good serological markers of infection. Infected women have IgG antibodies to MOMP, IncB and IncC and IgG2 (a Th1 dependent subclass) is the prevalent subclass of anti-Inc antibodies[11\*]. Antibody titres to IncB and IncC correlate with cervicitis and PID being highest in cervicitis[11\*]. CD4-T-cell responses to IncB and IncC in infertile women generated a Th2 cytokine pattern whilst fertile women showed a Th1 pattern[8\*].

Ct heat shock proteins (cHSPs) are analogous to human HSPs and antibodies to cHSP60 are associated with autoimmunity[41\*]. Ct STI is associated with antibodies to sperm in semen and IgG antibodies to cHSP60 are associated with reproductive failure. Sperm have several surface HSPs and cross-reaction with cHSPs may initiate autoimmune responses to sperm[42\*]. IgG responses to cHSP60 does not correlate with pathology in women[43\*] but women with tubal infertility have a high prevalence of antibodies to cHSP60[44\*].

Rank and Whitum-Hudson[45\*] reviewed experimental evidence for protective acquired immunity. In guinea-pigs antibody protects against reinfection and serum IgG is more important than local IgA. In mice both Th1 cells and antibody contribute directly to protection. MOMP is immunodominant, but MOMP immunization only protects if the protein is non-reduced, emphasizing the importance of native-protein B cell epitopes. Antibiotic therapy clears experimental infection but prevents generation of immunity[45\*].

## Vaccination

Important considerations for vaccination include: who to vaccinate; which antigen(s) to use; which administration route to use; and, which adjuvant to use.

There are two stages in the Ct lifecycle which a prophylactic vaccine should 'hit': 1) cell-adhesion, for which antibody would be most useful and 2) early-mid replication where T-cells might be targeted against bacterial antigens in infected cell membranes. Both responses would require CD4-T-cell activation. Acquired immunity is polarized by the antigen presenting cells (usually local dendritic cells/DCs) which first activate CD4-T-cell responses. Thus adjuvant activation of DCs local to the vaccination site is crucial. It should be remembered that although animal studies establish general principles, the fine details of immunity differ between species, and, although Ct is closely related to the widely used

murine pathogen *C. muridarum* (which is, somewhat confusingly, also referred to as the mouse pneumonitis strain of *C. trachomatis*) their biology is not identical.

A strong IFN- $\gamma$  response of lymphocytes to Ct antigen correlates with resistance to infection among sex workers[46\*]. An immunoproteomic analysis of *C. muridarum* indicates the two most likely vaccine candidates were PmpG and PmpE/F which several mouse strains recognise. However, peptides of these proteins used to vaccinate one mouse strain are not recognized by others arguing for whole protein rather than peptide vaccination. CpG oligonucleotide adjuvant does not induce protection perhaps due to lack of Th17 induction[46\*]. The balance between Th1/Th17 responses explains different susceptibilities of mouse strains to *C. muridarum*[47\*]. MOMP administered in Lipid-C adjuvant both transcutaneously[48\*] and orally[49\*] induces protection against vaginal *C. muridarum* which is enhanced via the oral route when CpG oligonucleotides and cholera toxin are incorporated into the Lipid-C[49\*]. CT043, a conserved Inc protein, is a CD4-T-cell target and may be a vaccine candidate[50\*]. Ct ArtJ [a protein conserved among chlamydial species] may also be a vaccine candidate with the additional benefit of also generating protection against the related human lung pathogen *C. pneumoniae*[51\*\*]. CPAF induces protection against vaginal *C. muridarum* which is boosted with IL-12 or CpG oligonucleotides as adjuvant[52\*]. Live infection induces Th1 responses whilst immunization with inactivated organisms induces Th2 responses[53\*]. TARP is an immunodominant protein and protects mice against *C. muridarum* infection[54\*].

The human Ct “antigen-ome” in sera from 99 infected women is very broad as over 700 Ct proteins were recognized by more than one serum[55\*\*]. Mice given either live CT infection or immunized with dead organisms were used to identify infection dependent and independent antigens which were tested against sera from infected women. The most recognized infection dependent protein (by 94% of sera) is CPAF. The most recognized infection independent protein (by 96% of sera) is Pgp3[55\*\*]. Use of serologically recognized proteins to identify CD4-T-cell epitopes also identified CPAF as a widely recognized serological antigen although in this series CT875 was the most commonly recognized protein[56\*\*]. MOMP is the most widely recognised T-cell epitope but CT875 stimulates most combined T-cell and serum responses[56\*\*].

## Concluding remarks

Understanding the natural history of infection, the host immune response and how these impact on subsequent pathology is crucial to rational vaccine design. Prophylactic vaccination aims to protect before infection. Therapeutic vaccination aims to boost or alter the immune response generated in previously infected individuals. There can be no doubt that vaccines against Ct would be of benefit to health and are likely to have a significant impact on healthcare costs. More work on identifying and testing vaccine candidates and appropriate delivery systems is needed.

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## Abbreviations

<b>CFTR</b>	cystic fibrosis transmembrane conductance regulator protein
<b>ChlaDub1</b>	Chlamydial Deubiquitinase and deneddylase Dub1



<b>CPAF</b>	chlamydial protease/proteasome-like activity factor
<b>Ct</b>	<i>Chlamydia trachomatis</i>
<b>DC</b>	dendritic cell
<b>EB</b>	elementary body
<b>ELISA</b>	enzyme linked immunosorbent assay
<b>ERalpha</b>	estrogen receptor alpha
<b>HSP</b>	heat shock protein
<b>HSV</b>	herpes simplex virus
<b>IFN-<math>\gamma</math></b>	interferon- $\gamma$
<b>IgG2</b>	Immunoglobulin subclass G2
<b>LGV</b>	lymphogranuloma venereum
<b>IL</b>	interleukin
<b>iNOS</b>	inducible nitric oxide synthase
<b>LPS</b>	lipopolysaccharide
<b>MAPK /ERK</b>	mitogen activated protein kinase
<b>MHC</b>	Major Histocompatibility Complex (protein)
<b>MOMP</b>	major outer membrane protein
<b>NF-<math>\kappa</math>B</b>	nuclear factor kappa-B
<b>NOD-1</b>	nucleotide-binding oligomerization domain containing 1
<b>PAMP</b>	pathogen associated molecular pattern
<b>PDI</b>	protein disulfide isomerase
<b>PID</b>	pelvic inflammatory disease
<b>PRR</b>	pattern recognition receptor
<b>RAS</b>	RAt sarcoma GTPase
<b>RB</b>	reticulate body
<b>SRAP-1</b>	Steroid receptor RNA activator 1
<b>STI</b>	sexually transmitted infection
<b>TARP</b>	translocated actin recruiting phosphoprotein
<b>Th</b>	T helper lymphocyte
<b>TLR</b>	Toll-like receptor

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### Key Points

- *Chlamydia trachomatis* genital infection is associated with subsequent reproductive pathology and infertility
- Infection with *Chlamydia trachomatis* induces partial immunity at best
- Vaccination against *Chlamydia trachomatis* would significantly reduce disease burden and health care costs
- Protective immunity can be induced experimentally
- Human *Chlamydia trachomatis* vaccine candidates and adjuvants require further study